AD_____

GRANT NUMBER: DAMD17-94-J-4110

TITLE: Antibody Specificity to Oncogene-Derived Peptides for Breast Cancer Detection

PRINCIPAL INVESTIGATOR: Clemencia Pinilla, Ph.D.

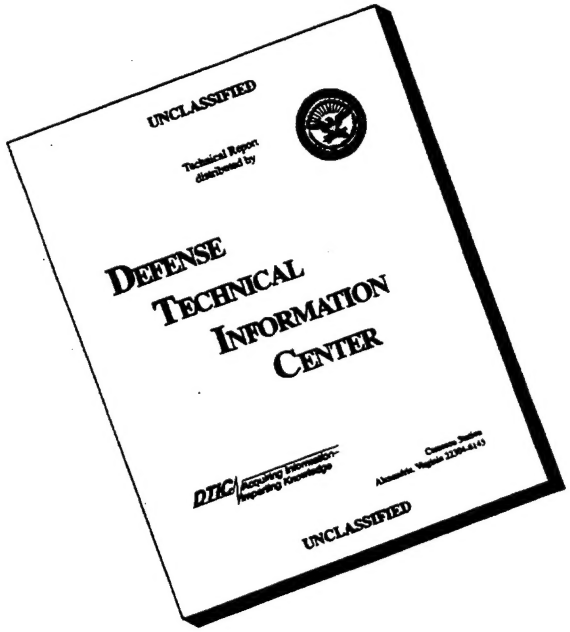
CONTRACTING ORGANIZATION: Torrey Pines Institute for Molecular Studies
San Diego, CA 92121

REPORT DATE: July 1996

TYPE OF REPORT: Annual

PREPARED FOR: Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, Maryland 21702-5012

DISCLAIMER NOTICE



THIS DOCUMENT IS BEST QUALITY AVAILABLE. THE COPY FURNISHED TO DTIC CONTAINED A SIGNIFICANT NUMBER OF PAGES WHICH DO NOT REPRODUCE LEGIBLY.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	July 1996	Annual (1 Jun 95 - 31 May 96)

4. TITLE AND SUBTITLE
Antibody Specificity to Oncogene-Derived Peptides for Breast Cancer Detection

5. FUNDING NUMBERS
DAMD17-94-J-4110

6. AUTHOR(S)
Clemencia Pinilla, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Torrey Pines Institute for Molecular Studies
San Diego, CA 92121

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, MD 21702-5012

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200*

This report describes the work carried out on the specificity of four oncogenic peptide-monoclonal antibody interactions. Monoclonal antibody 172-12A4, which was raised against a peptide from *v-erbB*, was assayed by ELISA with a set of 152 substitution analogs of the antigenic determinant to yield a fingerprint profile of this antigen-antibody interaction. A number of peptide libraries have also been screened and the deconvolution of individual sequences is ongoing. Peptide libraries have been screened against mAb 121-15B10, which was raised against a peptide from *v-fes* and is homologous with the *v-erbB* peptide. Peptides with nanomolar affinities have been identified. The antigenic determinants for six antibodies against two different oncogenic peptides were located using omission analogs. Substitution analogs are being synthesized to identify the amino acid specificities of each of these antibodies. Peptide libraries were also screened against these two peptide-antibody interactions, and the deconvolution process is ongoing. The understanding of antibody specificity at the amino acid level will help in the development of these antibodies as early diagnostics for breast cancer.

14. SUBJECT TERMS
Breast Cancer

15. NUMBER OF PAGES
38

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the US Army.

____ Where copyrighted material is quoted, permission has been obtained to use such material.

____ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

CP Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

____ In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

____ For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

____ In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

____ In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

____ In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

Clemencia Pinilla June 26, 1996.

PI - Signature Date

TABLE OF CONTENTS

Introduction	.....	5
Body	.....	6
Conclusions	.....	12
References	.....	13
Appendix:	Figure legends, figures 1-3, tables 1-6 and two papers that are in press.	

INTRODUCTION

It has been shown that many proteins encoded by oncogenes (*c-erbB-2)* and growth factor receptors (EGFR) are implicated in breast cancer. These proteins contain a consensus region that has homology with the catalytic core domain of protein kinases, and are involved in cell regulation and growth. The presence of these proteins in clinical samples of breast cancer patients has initiated numerous studies toward the development of antibody-based detection methods for early and effective diagnosis and prognosis of breast cancer. Peptide-specific monoclonal antibodies have also been shown to be able to detect these proteins in patient serum and/or urine samples, thus offering the potential for the ready detection and monitoring of the progression of the disease.

This study will focus on the elucidation of the specificities of a number of monoclonal antibodies raised against four different synthetic peptides derived from homologous regions of oncogene proteins. The potential use of these antibodies as specific probes to detect breast cancer-related protein markers in patient serum samples is under study (collaboration with Dr. Niman at the Univ. of Pittsburg). To further develop detection strategies using these antibodies, it is essential that their binding characteristics be determined at the amino acid level. The specificity at the amino acid level of these peptide-specific antibodies can be readily determined using complete sets of substitution analogs prepared by the method of simultaneous multiple peptide synthesis (SMPS) (1). When assayed against monoclonal antibodies by competitive ELISA, sets of substitution analogs provide a relative rank order of importance for each of the individual amino acids being substituted at a given position of an antigenic determinant, and also for each position making up the antigenic determinant (2,3).

Peptide libraries composed of millions of peptide sequences offer a unique advantage to fully understand the recognition capabilities of a monoclonal antibody for different peptide sequences. Synthetic combinatorial libraries (SCLs) offer the advantage of using nonsupport-bound peptides in relevant quantities needed for solution assays (first described in 4 and 5, and reviewed in 6). In this manner, we will determine the extent of crossreactivity of these antibodies for other oncogene-derived proteins and functionally-related proteins that share these sequences with protein kinases.

The antibody recognition patterns derived from this study will further our understanding of these specific interactions at the amino acid level, and will offer new approaches, such as using combinations of monoclonal antibodies, for the identification of protein products derived from oncogenes responsible for breast cancer. The use of well-characterized monoclonal antibodies will lead to earlier and more precise detection of relevant proteins in patient samples. These antibodies can also be used to monitor the progression of the disease in those cases where intervention and treatment may be necessary.

This report summarizes the work carried out from June 1, 1995 to May 31, 1996.

Monoclonal antibodies were assayed by ELISA against peptide omission and substitution analogs and peptide libraries. Information regarding the detailed specificities of these interactions at the amino acid level will be presented. Portions of this work have been published (7,8).

BODY

Peptide libraries and synthesis

A number of peptide libraries were screened against each monoclonal antibody (mAb), including the dual-defined libraries, hexa- and decapeptide positional scanning libraries, length libraries, and a number of nonpeptide libraries. Individual analogs and defined sequences deconvoluted from the various libraries were synthesized by SMPS.

Direct ELISA

Direct ELISA was used to assay the substitution analogs for antibody recognition (2,3). Peptides were adsorbed to microtiter plates at concentrations of 0.2 μM and 2 μM per well by incubation in 0.06 M sodium bicarbonate/0.03 M sodium carbonate buffer, pH 9.3, overnight at room temperature in a moist box. The plates were washed 10 times with deionized water to remove unbound peptide. Nonspecific absorption of mAb was blocked by incubating the plates with 100μl of 1% bovine serum albumin (BSA) (Sigma) in phosphate buffered saline (PBS) for 1 hour at 37° C. MAb was diluted with 1% BSA/PBS to give a maximum optical density (O.D.) reading for the original peptide, serially diluted down the plate, and then incubated at 37° C for 1 hr or 4°C overnight. Plates were washed as before. Horseradish peroxidase-conjugated goat antimouse IgG (Calbiochem) diluted with 1% BSA/PBS to 1:5000 50μl/well was added and the plates were incubated at 37° C for 1 hour. Excess antibody-conjugate was removed by washing as before. The amount of antibody-conjugate bound in each well was quantitated by reaction for 10 minutes with 50μl/well of freshly prepared developing solution consisting of o-phenylenediamine (Sigma) and 25μl of 3% hydrogen peroxide in 6ml of deionized water. The enzymatic reaction was stopped with 25μl/well of 4N sulfuric acid, and the resulting color was read at 492nm by a Titertek Multiscan spectrophotometer. The recognition of each peptide analog is expressed as a percentage of the mAb binding to the original peptide. Each peptide analog was assayed a minimum of three times.

Competitive ELISA

Competitive ELISA permits a more accurate quantification of the relative binding of peptide analogs or peptide libraries to mAb and lessens undesirable effects caused by differential binding of individual peptide analogs to the ELISA plate (3). Competitive ELISA is carried out in a similar manner as the direct ELISA described above, with the following modifications: 1) only the control peptide is bound to the plate at an optimal concentration; and 2) serial concentrations of each peptide analog or peptide mixture are added before the addition of an optimal concentration of specific mAb. The

concentration of each analog or peptide mixture necessary to inhibit 50% of the binding of the mAb to the original peptide on the plate was determined (IC_{50}) using Graphpad (ISI Software). To determine the relative replaceability of each position of the antigenic determinant from the substitution analog results, the IC_{50} of each peptide analog was divided by the IC_{50} of the control peptide to yield the replaceability factor (RF) for each peptide analog. Individual RFs are a relative measure of each analog's ability to inhibit mAb binding as compared to the control peptide, which has an RF of 1.0. Thus, an analog with an RF less than 1.0 is one for which a lower concentration of analog is needed to inhibit 50% of mAb binding to the control peptide, and vice versa. To determine the overall replaceability of each position within the antigenic determinant, the average of the RFs of the 19 substitution analogs at each position is termed the relative positional importance factor (RPIF). This number represents the relative functional contribution to mAb binding of each position within the antigenic determinant.

Results

The results are presented for each of the four antigen-antibody interactions. The progress of each task outlined in the proposal is briefly summarized below:

Task 1: Synthesis of substitution analogs
The substitution analogs for LGSGAFGTIYKG/mAb 172 and half of the substitution analogs for IGRGNFGEVFSGC/mAb 121 interaction have been made. Synthesis of substitution analogs for the IMVKCWMIDADSRPKF/mAb 171 interaction is underway. The remaining substitution analogs for IGRGNFGEVFSGC and the LMEQCWAYEPGQRPSF/mAb 127 interaction will be completed soon.

Task 2: Assaying of peptide-antibody interactions
Direct and competitive ELISA results have been completed for the LGSGAFGTIYKG/mAb 172 interaction. Competitive conditions have been established for the other three interactions. Direct and competitive ELISA will be carried out when peptide analogs are finished.

Task 3: Screening of peptide libraries
Library screening is ongoing for all four interactions. Details are provided below for each peptide-antibody interaction.

Task 4: Identification of defined peptides from library screening
Defined peptides have been synthesized and assayed in their respective peptide-antibody interactions. Individual peptides have already been identified for two of the four interactions. More details are provided below for each peptide-antibody interaction.

LGSGAFGTIYKG/mAb 172-12A4

Substitution analog profile

The final ELISA data (direct and competitive) for each of the 152 substitution analogs of the antigenic determinant -AFGTIYKG- of the peptide LGSGAFGTIYKGC (from residues 138-149 of *v-erbB*) recognized by mAb 172-12A4 is shown in Figure 1. From the direct ELISA data, the substitution profile revealed that mAb 172-12A4 recognized a discontinuous linear determinant, in which four residues (F, G, I, K) were specific and four residues were relatively redundant. Interestingly, glycine appeared twice in the antigenic determinant, each playing a different role. At position 3 of the antigenic determinant, glycine was highly specific, but the same amino acid at position 8 was redundant. This difference in specificity was also found for phenylalanine at position 2 (specific) and tyrosine, which is often a conservative replacement of phenylalanine, at position 6 (redundant).

Competitive ELISA data is shown below the bar graphs at each position of the antigenic determinant. The concentration necessary to inhibit 50% (IC_{50}) of mAb 172-12A4 binding to the control peptide adsorbed to the plate was determined for each substitution analog. The replaceability factor (RF) represents a relative measure of each analog's ability to inhibit mAb 172-12A4 as compared to the control peptide (RF=1.0). The data agrees with the direct ELISA data in that several positions were relatively redundant and several positions were specific. However, the competitive ELISA yields a more quantitative depiction of mAb 172-12A4 recognition with each residue of the antigenic determinant. For example, the majority of analogs for three redundant positions, namely the C-terminal glycine, tyrosine, and threonine, had IC_{50} values that were slightly lower than the control peptide. The alanine position was relatively specific in that only serine and valine had IC_{50} values comparable with the control peptide. Isoleucine was also relatively specific since only conservative substitutions such as valine and leucine, along with methionine and proline, were recognized. The absence of a side chain for the specific glycine position is highly critical for mAb recognition, as seen by the lack of recognition for the 19 substitution analogs. The majority of analogs for the remaining specific positions, namely lysine and phenylalanine, were poorly recognized (>50-fold) by mAb 172-12A4, except those analogs that represented conservative substitutions. The arginine substitution analog for lysine was nearly 7-fold less active than the control, whereas the tyrosine substitution analog for phenylalanine had nearly the same IC_{50} value as the control peptide.

The overall replaceability of a given position in the antigenic determinant was determined by averaging the IC_{50} values for the 19 analogs at each position. This averaged IC_{50} value is termed the relative positional importance factor (RPIF). An RPIF of 1.0 indicates that there is no difference in recognition for the 19 substitution analogs relative to the control peptide (IC_{50}=180nM). A high RPIF represents a relatively specific position. It was found that mAb 172-12A4 recognized a discontinuous linear determinant, in which three residues were highly specific, namely lysine (RPIF=112),

phenylalanine (RPIF=105), and glycine (RPIF=61). Two positions were moderately specific, namely isoleucine (RPIF=14) and alanine (RPIF=12), and three positions were relatively redundant, namely threonine (RPIF=4.3), tyrosine (RPIF=1.1) and glycine (RPIF=0.9). From previous studies (3), a good correlation has been found between residues that are irreplaceable for mAb recognition and residues that make contact with specific residues of the mAb. Thus, mAb 172-12A4 may be able to recognize sequences from other protein kinases that maintain these specific residues, but differ at the redundant positions.

Positional scanning library

The positional scanning hexapeptide library screening data yielded the most active amino acid residues at each position for a hexapeptide sequence. Individual peptides were made from the results of the two sublibraries prepared. The most active peptides were found to be analogs of the antigenic determinant, although their activities were lower, suggesting that a sequence longer than six residues is required for high affinity binding. Analogs have been made by adding each of the 20 amino acids to the best hexamer (YGPIDK) at the N-terminus (OYGPIDK) and C-terminus (YGPIDKO).

Decapeptide library screening

The screening profile for the decapeptide positional scanning library revealed similar results as the hexapeptide library, in which only the specific residues displayed significant inhibition at several positions of the decapeptide (Table 1). Even with a stringent selection criteria, two to five amino acids were chosen at each position, resulting in an large number of peptides to make (92,160 different combinations). An alternative approach would involve the synthesis of mixtures having two positions defined with the most active amino acids found from the screening data will be prepared (Table 2). In this manner, the connectivity between two positions will be established before individual peptides are made. For example, alanine and tyrosine were chosen in position 1 and glycine and tyrosine were chosen in position 2. The four possible combinations for these two positions will be made resulting in four peptide mixtures, i.e., Ac-AGXXXXXXXX-NH_2, Ac-AYXXXXXXXX-NH_2, Ac-YGXXXXXXXX-NH_2, and Ac-YYXXXXXXXX-NH_2. The same will be done for amino acids defined in positions 3 and 4, 5 and 6, 7 and 8, and 9 and 10. A different frame will also be used to make mixtures, namely positions 2 and 3, 4 and 5, 6 and 7, 8 and 9, and 10 and 1. A total of 108 mixtures will be prepared, which is a more practical number to make than the individual combinations chosen from the screening data. This approach is expected to minimize the overall number of sequences that would have to be synthesized as well as identify only those sequences with the highest activities. Once the connectivity is determined from these two sets of iterations, individual peptides will then be prepared.

Dual-defined library

Each of the dual-defined hexapeptide SCLs is composed of 400 peptide

mixtures, having two positions defined and four positions as mixtures. Thus, 1200 different peptide mixtures were screened by competitive ELISA and their IC_{50} values were determined. Residues -YG- are the most active residues in positions 1 and 2, and lysine is clearly the most active residue in position 6. There is some overlap of -YG- in positions 3 and 4. However, the isoleucine residue, which is the expected residue in position 4 of the antigenic sequence, is found in several active peptide mixtures. The connectivity of the most active amino acids from the three different libraries will be determined by synthesizing individual peptides representing the combinations of the most active peptide mixtures.

Other libraries

Several peptidomimetic and nonpeptide libraries were screened for inhibition of mAb 172-12A4 binding to the antigenic peptide LGSGAFGTIYKG. These four different libraries, namely per-allylated, per-benzylated, per-benzylated amine, and amine, were chemically modified from the same positional scanning tetrapeptide library composed of 52 different L-, D-, and unusual amino acids. Little activity was found in each screen, suggesting that the peptide bonds, which were chemically removed in these libraries, as well as the length (tetramers vs. hexamers and decamers) are essential for mAb recognition.

IGRGNFGEVFSGC/mAb 121-15B10

Length library

The screening of the length SCL (OXX-NH_2 up to OXXXXXXXXX-NH_2) revealed that isoleucine, and to a lesser extent methionine, were the most active amino acids in the first position of sequences ranging from 6-9 residues in length. A hexapeptide positional scanning SCL was screened, and based on the screening results, individual peptides resulting from the most active amino acids were prepared and assayed. The first three residues of the most active peptides corresponded with the immunogen sequence (-IGR-).

Dual-defined library

Next, three different dual-defined SCLs were screened (Figure 2A-C). Position 1 was specific for isoleucine, methionine, and leucine, and position two was specific only for glycine. Positions 3 and 4 were specific only for arginine. Peptide mixtures having the fifth and sixth positions defined were less active, yet several amino acids were chosen. Based on the IC_{50} values of the most active mixtures, five individual peptides were prepared and tested. The most active peptide was IGRRNM-NH_2 (IC_{50}=30nM, Table 3), in which four of the six residues (IGR_N_) correlate with the immunogen.

The iterative process was also carried out for the most active peptide mixture from the three dual-defined SCLs, namely XXRRXX-NH_2. Using a bidirectional iterative approach, the first set of iterations defined the second and fifth positions. Upon screening these iterations, glycine was clearly the only amino acid active at position 2.

Tyrosine and other aromatic amino acids, as well as asparagine, aspartic and glutamic acid, were the most active at position 5, although their activities were only three-fold better than XXRRXX-NH_2. The next set of iterations defined the first and sixth positions of XGRRYX-NH_2. Isoleucine and methionine were the most active in position 1, whereas tyrosine and several other amino acids were active in position 6. Final sequences were made from the iterative screening data and assayed (Table 4). The most active sequences contained the following motif: (I/M) G R R (Y/E) (Y/L/M). The first four residues of this motif were found to be the most specific for this peptide-antibody interaction. These results correlate well with those found from screening the three dual-defined SCLs, indicating that differently formatted libraries representing the same diversities will yield similar results.

Since the length library resulted in peptide mixtures of 6-9 residues that were the most active, heptapeptide analogs were prepared for IGRRYY-NH_2 in an effort to improve its activity. Each of the 20 L-amino acids was added to the C-terminus and assayed. A two- to three-fold increase was found for the most active peptide IGRRYYD-NH_2 (IC_{50}=9nM, Table 5).

IMVKCWMIDADSRPKF/mAb 171

Competitive ELISA conditions were optimized for three antibodies, namely 19B10, 11B9, and 10E5, against this peptide. The IC_{50} values of the control sequence were 30nM for 19B10, 10nM for 11B9, and 50nM for 10E5. Omission analogs were assayed by competitive ELISA to identify the antigenic determinants recognized by three monoclonal antibodies from the 171 series The antigenic determinants center around residues -MIDADSR- (Table 6). Substitution analogs will be prepared for this region and assayed by direct and competitive ELISA.

The first SCLs tested were the length library followed by the positional scanning hexapeptide library. The screening profile from the hexapeptide library against mAb 171-19B10 is shown in Figure 3. Significant inhibition was found at each position by amino acids corresponding to the antigenic determinant. Combinations will be made based on the peptide mixtures that show greater than 40% inhibition at more than one library concentration. A similar screening profile was found for mAb 171-10E5, which recognizes the same antigenic determinant as mAb 171-19B10. The screening profile for mAb 171-11B9 was not as definitive. The dual defined hexapeptide libraries will be tested to confirm the positional scanning library screening for mAb 171-19B10 and 10E5, as well as to identify sequences to make for mAb 171-11B9.

LMEQCWAYEPGQRPSF/mAb 127

Competitive ELISA conditions were determined for three different antibodies from the mAb 127 series, namely 53F8, 42C11, and 50D4. The IC_{50} values for the control peptide were 15nM for 53F8, 1000nM for 42C11, and 300nM for 50D4. Omission analogs were also assayed, and the antigenic determinants were located

near residues -WAYEPGQR- for all three antibodies (Table 6). Substitution analogs will be made for this region and assayed by direct and competitive ELISA.

The positional scanning hexapeptide library was screened against all three libraries. No definitive profiles could be obtained. The dual defined libraries will be tested next, and individual peptides will be prepared based on the activities of the best mixtures.

CONCLUSIONS

The preliminary mapping of these four peptide-antibody interactions has been carried out using substitution analogs and/or peptide libraries. The amino acid level of specificities found for these interactions is required for the use of monoclonal antibodies in the detection of oncogene-derived products. Early detection of these products implicated in breast cancer will lead to earlier treatment of this disease.

Future experiments

For mAb 172-12A4, Individual hexapeptides representing combinations of amino acids from the dual-defined libraries will be prepared and assayed to finish the hexapeptide library data. Iterations will be made from the decapeptide library results in order to identify longer sequences with high activities. Peptide libraries composed of D- and unusual amino acids, will be screened against LGSGAFGTIYKG/mAb 172-12A4 for the potential identification of unrelated sequences recognized by this mAb. The final sequences identified from the library screenings will be used to search a sequence database for homology with other protein sequences.

The substitution analogs for IGRGNFGEVFSGC will be finished and tested against a number of monoclonal antibodies from the mAb 121 series. Also, more libraries will be assayed against these monoclonal antibodies.

Synthesis of the substitution analogs for the remaining two peptides is underway. Initial results from the screening of peptide libraries is encouraging, and more libraries will be screened before any deconvolution to individual sequences is made.

REFERENCES

1. Houghten, R.A. Proc. Natl. Acad. Sci., 82 (1985) 5131.
2. Appel, J.R., Pinilla, C., Niman, H., and Houghten, R.A. J. Immunol., 144 (1990) 976.
3. Pinilla, C., Appel, J.R., and Houghten, R.A. Mol. Immunol., 30 (1993) 577.
4. Houghten, R. A., Pinilla, C., Blondelle, S.E., Appel, J.R., Dooley, C.T., and Cuervo, J.H. Nature, 354 (1991) 84.
5. Pinilla, C., Appel, J.R., Blanc, P., and Houghten, R.A. BioTechniques, 13 (1992) 901.
6. Pinilla, C., Appel, J.R., Blondelle, S., Dooley, C.T., Dörner, B., Eichler, J., Ostresh, J., and Houghten, R.A. Biopolymers (Peptide Science), 37 (1995) 221.
7. Appel, J.R., Buencamino, J., Houghten, R.A., and Pinilla, C. In Peptides: Proceedings of the 14th American Peptide Symposium, in press.
8. Appel, J.R., Buencamino, J., Houghten, R.A., and Pinilla, C. Mol.Diversity, in press.

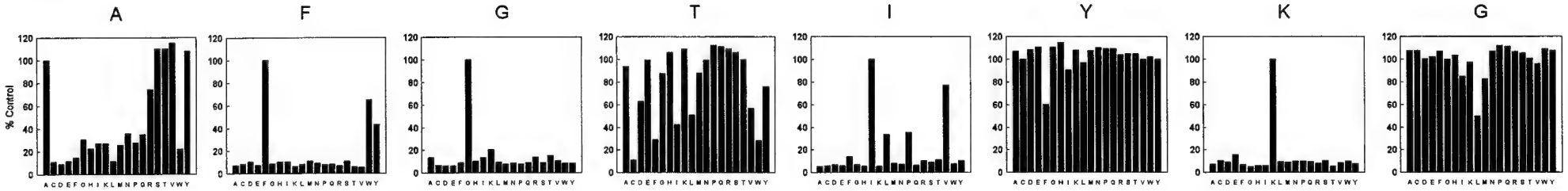
Figure legends

Figure 1. LGSGAFGTIYKG/mAb 172-12A4. Top. Direct ELISA substitution profiles for each position of the antigenic determinant (-AFGTIYKG-) of LGSGAFGTIYKG. The recognition of each substitution analog by mAb 172-12A4 is represented as the relative percentage of mAb which binds to the original peptide. Analogs for each position are represented on the *x*-axis in alphabetical order using the single-letter amino acid code. Botton. Competitive ELISA data for each substitution analog is expressed as its IC_{50} (μM) and replaceability factor (RF). Each position is ranked by RF from lowest to highest. The original residue at each position is outlined.

Figure 2. A. Screening of 400 mixtures of a SCL represented as OOXXXX-NH_2 . The activity of each peptide mixture is expressed as percent inhibition of mAb 121-15B10 binding to the antigenic peptide IGRGNFGEVFSGC-NH_2. Amino acids making up the second position of the mixture are on the *x*-axis of each bar graph in alphabetical order using the single-letter code. B. Screening of 400 mixtures of a SCL represented as XXOOXX-NH_2. C. Screening of 400 mixtures of a SCL represented as XXXXOO-NH_2.

Figure 3. Screening of the hexapeptide PS-SCL for the ability to inhibit mAb 171-19B10 to the antigenic peptide IMVKCWMIDADSRPKF-NH_2. Each panel represents the percent inhibition of the peptide mixture for a given positional SCL. Amino acids in the defined positions are represented on the *x*-axis in single-letter code.

Figure 1



AA	IC50	RF
A	180	1.0
S	454	2.5
V	665	3.7
Y	821	4.6
T	861	4.8
F	999	5.6
Q	1224	6.8
H	1271	7.1
D	1305	7.3
I	1356	7.5
R	1644	9.1
N	1658	9.2
P	1745	9.7
E	2375	13.2
C	2702	15.0
M	2750	15.3
G	2773	15.4
K	2866	15.9
L	3321	18.4
W	9150	50.8
RPIF		12

AA	IC50	RF
F	180	1.0
Y	190	1.1
C	1120	6.2
M	1400	7.8
H	1700	9.4
W	5400	30.0
L	12800	71.1
R	13300	73.9
E	14150	78.6
I	14800	82.2
N	16200	90.0
G	18500	102.8
V	24550	136.4
P	26450	146.9
S	27650	153.6
T	28400	157.8
Q	32850	182.5
K	37000	205.6
A	40000	222.2
D	41000	227.8
RPIF		105

AA	IC50	RF
G	180	1.0
P	1200	6.7
N	1635	9.1
H	2400	13.3
D	2500	13.9
S	2750	15.3
Q	2950	16.4
Y	3150	17.5
E	3850	21.4
A	5350	29.7
R	6250	34.7
K	7750	43.1
C	10300	57.2
T	11700	65.0
F	13050	72.5
M	14250	79.2
W	21000	116.7
I	31500	175.0
V	32000	177.8
L	35500	197.2
RPIF		61

AA	IC50	RF
P	27	0.2
K	77	0.4
E	91	0.5
Q	107	0.6
A	125	0.7
R	138	0.8
T	180	1.0
S	213	1.2
D	229	1.3
L	257	1.4
V	259	1.4
I	397	2.2
N	462	2.6
M	501	2.8
G	544	3.0
H	647	3.6
C	959	5.3
F	1727	9.6
Y	2185	12.1
W	5853	32.5
RPIF		4.3

AA	IC50	RF
I	180	1.0
P	569	3.2
M	830	4.6
V	850	4.7
L	883	4.9
H	950	5.3
G	980	5.4
Q	1390	7.7
D	1400	7.8
Y	1450	8.1
T	1544	8.6
C	1580	8.8
S	1640	9.1
F	1850	10.3
E	2400	13.3
A	2450	13.6
N	3150	17.5
W	4900	27.2
K	8050	44.7
R	11800	65.6
RPIF		14

AA	IC50	RF
D	27	0.2
E	31	0.2
N	47	0.3
S	58	0.3
A	62	0.3
K	77	0.4
T	79	0.4
C	80	0.4
P	85	0.5
R	94	0.5
V	101	0.6
Q	102	0.6
H	127	0.7
M	129	0.7
W	141	0.8
F	155	0.9
G	155	0.9
I	167	0.9
Y	180	1.0
L	2074	11.5
RPIF		1.1

AA	IC50	RF
K	180	1.0
R	1231	6.8
P	1300	7.2
Q	3000	16.7
G	3900	21.7
E	4050	22.5
S	4550	25.3
H	5050	28.1
D	5050	28.1
N	5600	31.1
W	6950	38.6
A	12150	67.5
C	15100	83.9
T	23500	130.6
L	26750	148.6
F	30800	171.1
V	33500	186.1
M	57000	316.7
Y	70000	388.9
I	72500	402.8
RPIF		112

AA	IC50	RF
T	44	0.2
S	50	0.3
P	79	0.4
V	100	0.6
N	105	0.6
Q	119	0.7
Y	120	0.7
H	127	0.7
W	133	0.7
K	152	0.8
I	155	0.9
D	161	0.9
E	162	0.9
G	180	1.0
C	185	1.0
R	189	1.0
F	284	1.6
M	291	1.6
A	305	1.7
L	324	1.8
RPIF		0.9

Figure 2A

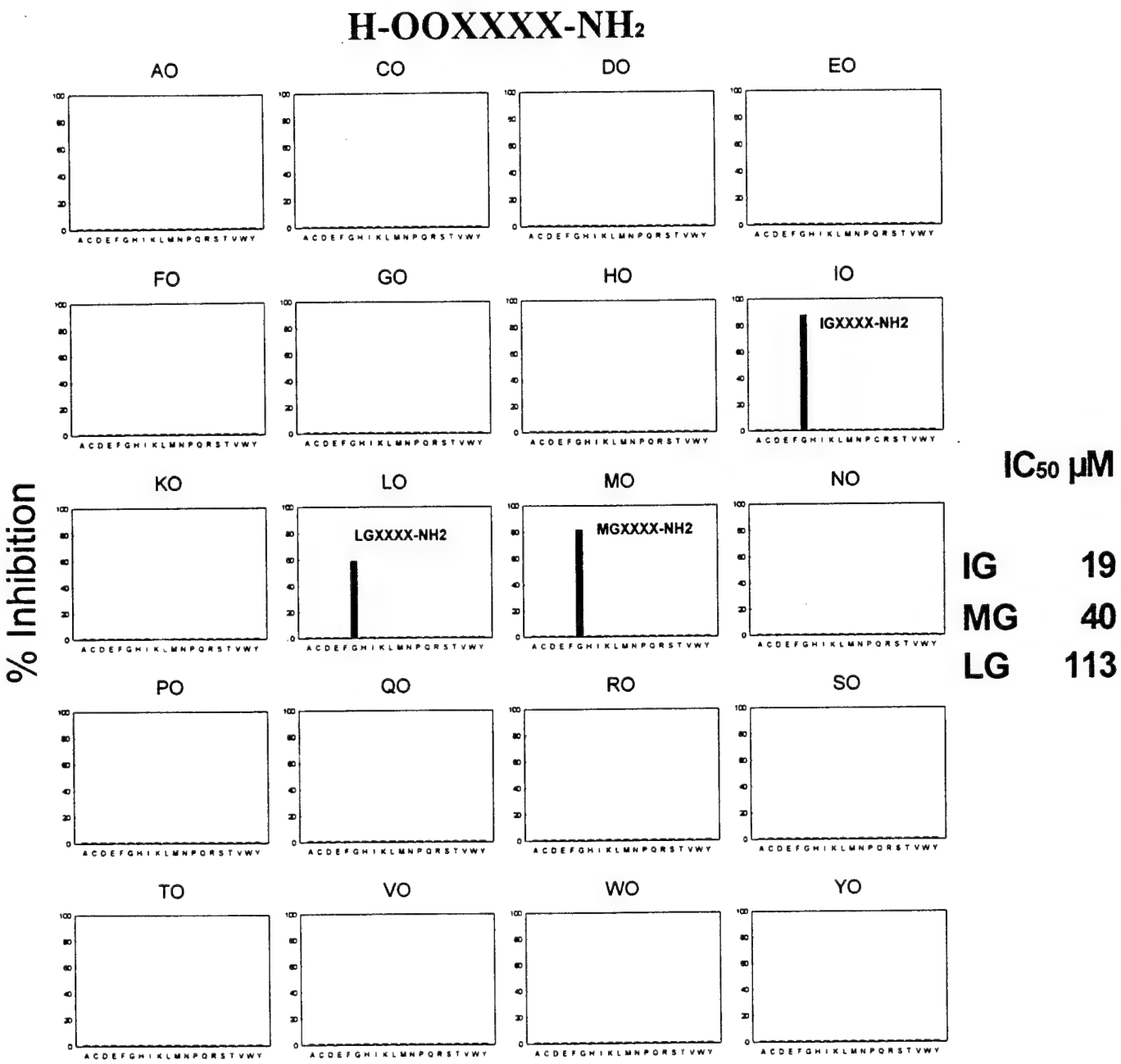

H-OOXXXX-NH2
AO
CO
DO
EO
FO
GO
HO
IO
IGXXXX-NH2
KO
LO
LGXXXX-NH2
MO
MGXXXX-NH2
NO
PO
QO
RO
SO
TO
VO
WO
YO
% Inhibition
A C D E F G H I K L M N P Q R S T V W Y
IC50 µM
IG 19
MG 40
LG 113

Figure 2B

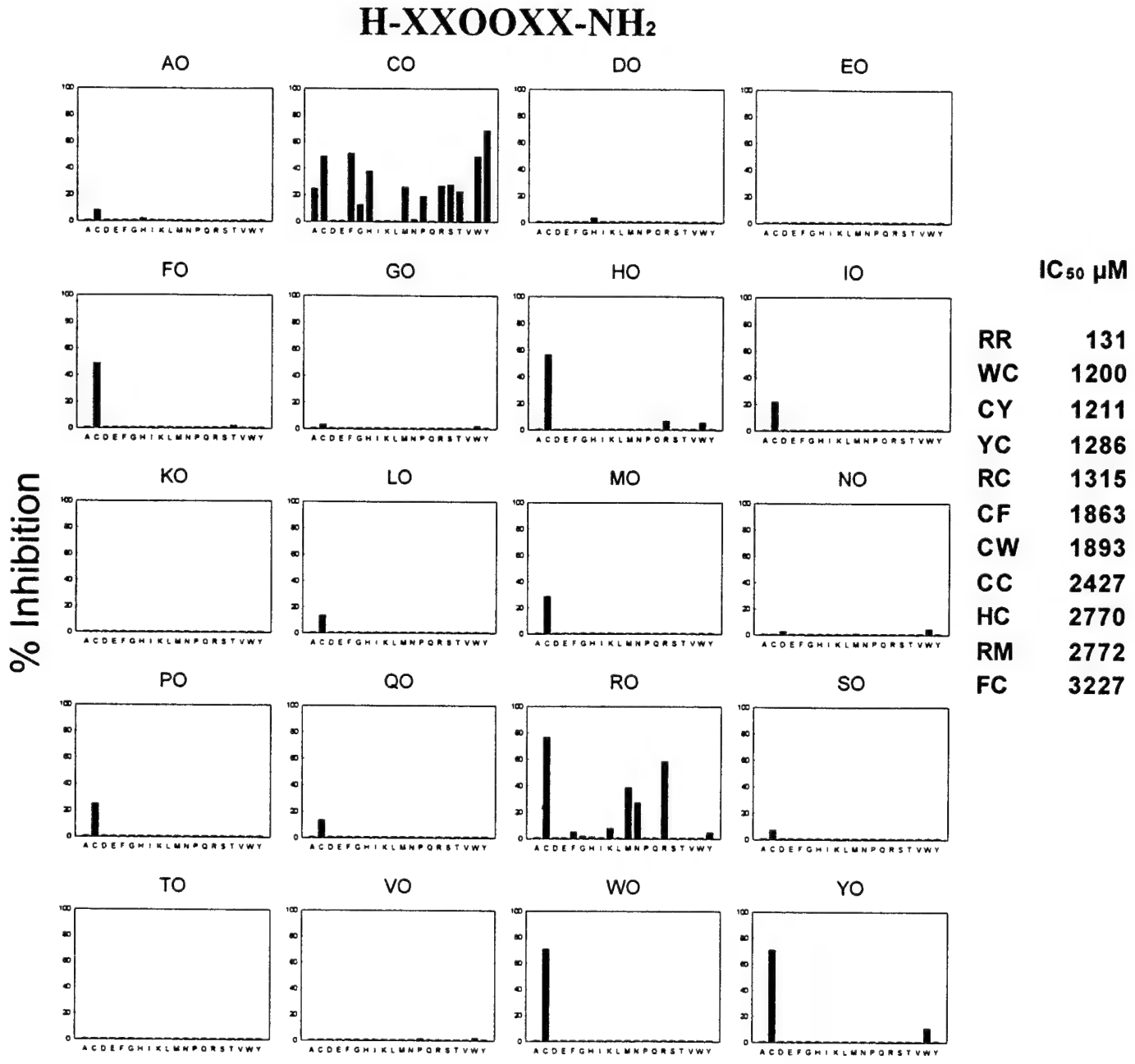

H-XXOOXX-NH2
% Inhibition
AO
CO
DO
EO
FO
GO
HO
IO
KO
LO
MO
NO
PO
QO
RO
SO
TO
VO
WO
YO
A C D E F G H I K L M N P Q R S T V W Y
IC50 µM
RR 131
WC 1200
CY 1211
YC 1286
RC 1315
CF 1863
CW 1893
CC 2427
HC 2770
RM 2772
FC 3227

Figure 2C

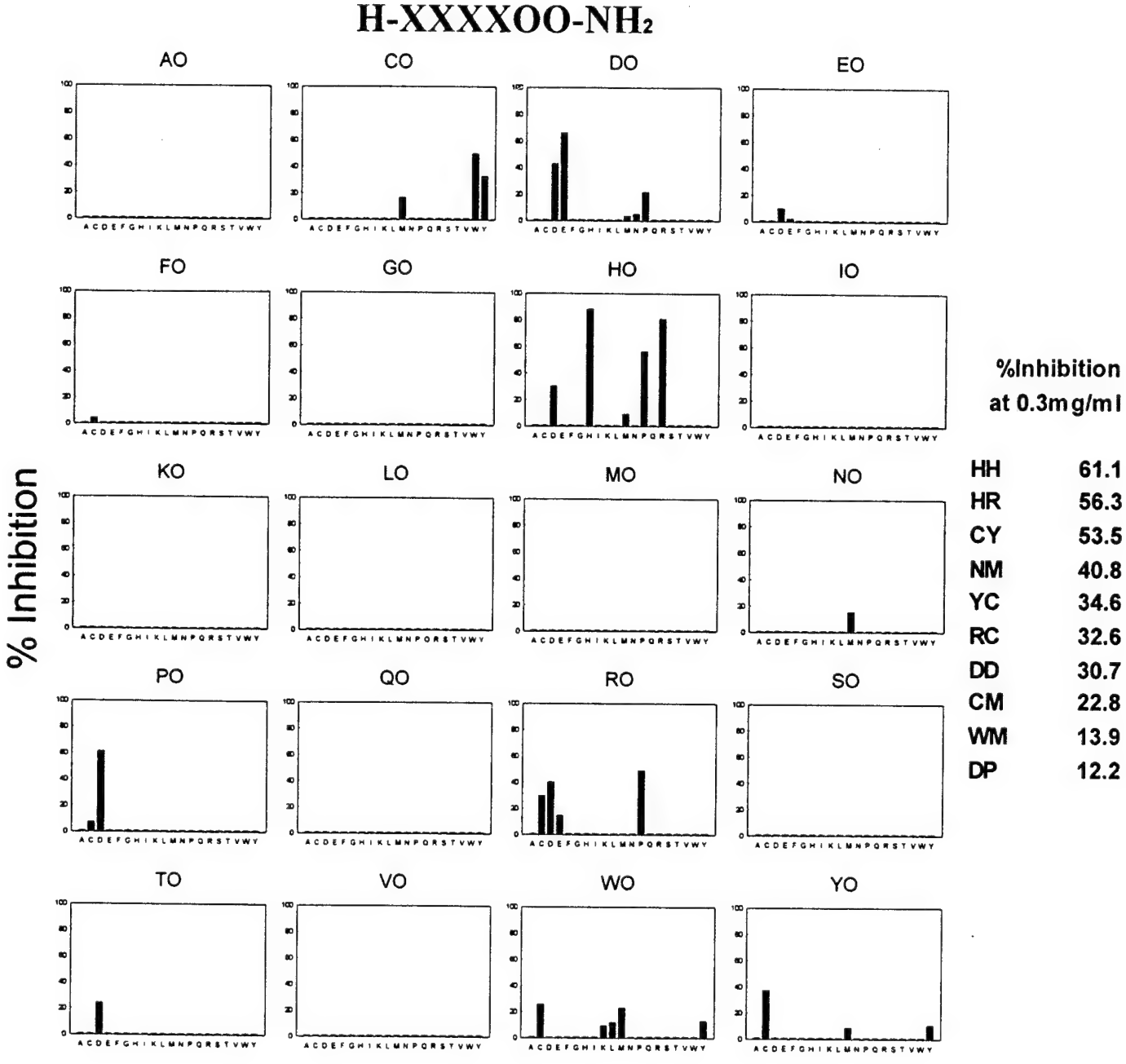

H-XXXXOO-NH2
AO
CO
DO
EO
FO
GO
HO
IO
KO
LO
MO
NO
PO
QO
RO
SO
TO
VO
WO
YO
A C D E F G H I K L M N P Q R S T V W Y
% Inhibition
%Inhibition at 0.3mg/ml
HH 61.1
HR 56.3
CY 53.5
NM 40.8
YC 34.6
RC 32.6
DD 30.7
CM 22.8
WM 13.9
DP 12.2

Figure 3

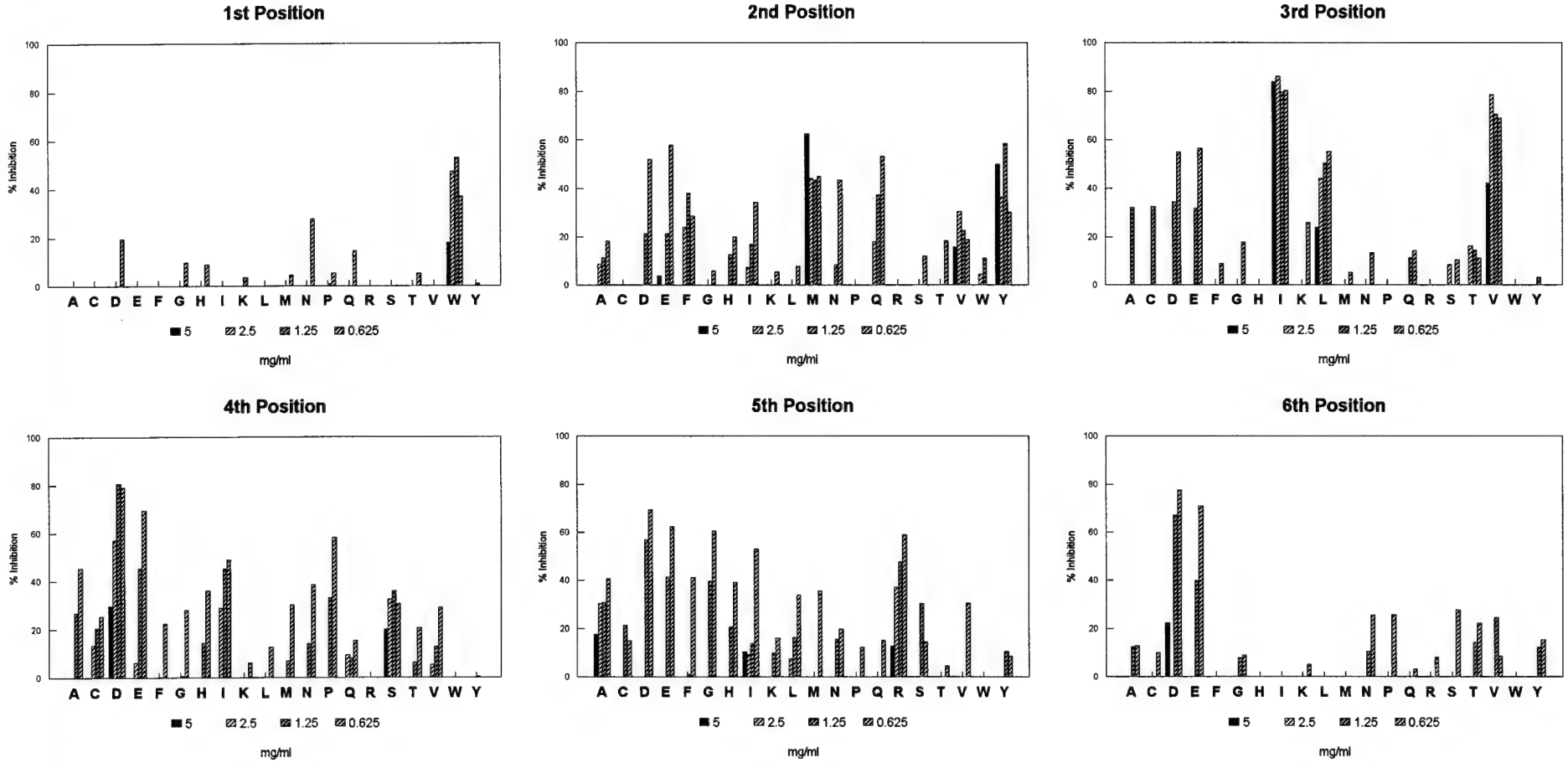

1st Position
2nd Position
3rd Position
4th Position
5th Position
6th Position
% Inhibition
A C D E F G H I K L M N P Q R S T V W Y
5
2.5
1.25
0.625
mg/ml

Table 1. IC_{50} values for mixtures of the decapeptide PS-SCL.

1st Position		2nd Position		3rd Position		4th Position		5th Position	
	IC_{50} µM		IC_{50} µM		IC_{50} µM		IC_{50} µM		IC_{50} µM
C	**104**	Y	**158**	C	**133**	C	**104**	C	**118**
Y	**145**	G	**205**	P	**407**	Y	**452**	D	**417**
A	**623**	C	**551**	G	**419**	G	**568**	G	**555**
D	**693**	A	**919**	Y	**517**	I	**659**	Y	**601**
S	**1105**	D	**1482**	D	**1405**	P	**809**	P	**782**
F	**1298**	S	**1738**	T	**3469**	A	**2768**	N	**826**
G	**2365**	F	**2456**	A	**4350**	S	**4350**	K	**2198**
T	**4163**	T	**4350**	N	**6282**	V	**4350**	A	**2425**
N	**4350**	N	**6525**	E	**6525**	T	**5889**	E	**3092**
E	**6525**	E	**8700**	K	**6525**	D	**6525**	S	**3319**
K	**6525**	H	**8700**	Q	**6525**	E	**8700**	I	**3666**
H	**8700**	I	**8700**	S	**6525**	F	**8700**	V	**5130**
I	**8700**	K	**8700**	F	**8700**	H	**8700**	T	**5212**
L	**8700**	L	**8700**	H	**8700**	K	**8700**	Q	**5367**
M	**8700**	M	**8700**	I	**8700**	L	**8700**	F	**6525**
P	**8700**	P	**8700**	L	**8700**	M	**8700**	H	**6525**
Q	**8700**	Q	**8700**	M	**8700**	N	**8700**	L	**8700**
R	**8700**	R	**8700**	R	**8700**	Q	**8700**	M	**8700**
V	**8700**	V	**8700**	V	**8700**	R	**8700**	R	**8700**
W	**8700**	W	**8700**	W	**8700**	W	**8700**	W	**8700**

6th Position		7th Position		8th Position		9th Position		10th Position	
	IC_{50} µM		IC_{50} µM		IC_{50} µM		IC_{50} µM		IC_{50} µM
C	**132**	C	**105**	C	**156**	C	**84**	C	**105**
G	**275**	K	**407**	K	**299**	K	**536**	S	**451**
K	**542**	D	**608**	I	**337**	D	**803**	K	**498**
D	**758**	P	**931**	G	**515**	Y	**1039**	P	**616**
Y	**1251**	G	**965**	D	**661**	V	**1528**	Y	**857**
P	**1591**	Y	**1039**	P	**841**	E	**1601**	D	**1072**
E	**2252**	I	**1325**	V	**1985**	P	**1653**	A	**1667**
F	**3263**	T	**1495**	Y	**2526**	I	**1709**	E	**1777**
I	**3453**	S	**2848**	E	**3018**	G	**2193**	G	**1845**
N	**6525**	V	**3561**	H	**5697**	T	**2306**	H	**1849**
S	**6525**	A	**4236**	A	**6054**	L	**3346**	I	**2044**
A	**8700**	E	**5438**	N	**6525**	H	**4350**	V	**2533**
H	**8700**	H	**5800**	S	**6525**	Q	**5706**	T	**3108**
L	**8700**	N	**6525**	F	**8700**	A	**6525**	Q	**3634**
M	**8700**	F	**8700**	L	**8700**	N	**6525**	N	**3750**
Q	**8700**	L	**8700**	M	**8700**	S	**6525**	L	**6214**
R	**8700**	M	**8700**	Q	**8700**	F	**8700**	F	**6525**
T	**8700**	Q	**8700**	R	**8700**	M	**8700**	W	**6525**
V	**8700**	R	**8700**	T	**8700**	R	**8700**	M	**8700**
W	**8700**	W	**8700**	W	**8700**	W	**8700**	R	**8700**

Table 2 Dual-defined peptide mixtures in two different frames from decapeptide library screening.

Position	1	2	3	4	5	6	7	8	9	10	
Amino acid	A	G	G	G	D	D	D	D	D	K	
	Y	Y	P	I	G	G	G	G	K	P	
			Y	P	P	K	K	I		S	
				Y	Y		P	K		Y	
								P			
1 Ac-	A	G	X	X	X	X	X	X	X	X	-NH2
2 Ac-	A	Y	X	X	X	X	X	X	X	X	-NH2
3 Ac-	Y	G	X	X	X	X	X	X	X	X	-NH2
4 Ac-	Y	Y	X	X	X	X	X	X	X	X	-NH2
5 Ac-	X	X	G	G	X	X	X	X	X	X	-NH2
6 Ac-	X	X	G	I	X	X	X	X	X	X	-NH2
7 Ac-	X	X	G	P	X	X	X	X	X	X	-NH2
8 Ac-	X	X	G	Y	X	X	X	X	X	X	-NH2
9 Ac-	X	X	P	G	X	X	X	X	X	X	-NH2
10 Ac-	X	X	P	I	X	X	X	X	X	X	-NH2
11 Ac-	X	X	P	P	X	X	X	X	X	X	-NH2
12 Ac-	X	X	P	Y	X	X	X	X	X	X	-NH2
13 Ac-	X	X	Y	G	X	X	X	X	X	X	-NH2
14 Ac-	X	X	Y	I	X	X	X	X	X	X	-NH2
15 Ac-	X	X	Y	P	X	X	X	X	X	X	-NH2
16 Ac-	X	X	Y	Y	X	X	X	X	X	X	-NH2
17 Ac-	X	X	X	X	D	D	X	X	X	X	-NH2
18 Ac-	X	X	X	X	D	G	X	X	X	X	-NH2
19 Ac-	X	X	X	X	D	K	X	X	X	X	-NH2
20 Ac-	X	X	X	X	G	D	X	X	X	X	-NH2
21 Ac-	X	X	X	X	G	G	X	X	X	X	-NH2
22 Ac-	X	X	X	X	G	K	X	X	X	X	-NH2
23 Ac-	X	X	X	X	P	D	X	X	X	X	-NH2
24 Ac-	X	X	X	X	P	G	X	X	X	X	-NH2
25 Ac-	X	X	X	X	P	K	X	X	X	X	-NH2
26 Ac-	X	X	X	X	Y	D	X	X	X	X	-NH2
27 Ac-	X	X	X	X	Y	G	X	X	X	X	-NH2
28 Ac-	X	X	X	X	Y	K	X	X	X	X	-NH2
29 Ac-	X	X	X	X	X	X	D	D	X	X	-NH2
30 Ac-	X	X	X	X	X	X	D	G	X	X	-NH2
31 Ac-	X	X	X	X	X	X	D	I	X	X	-NH2
32 Ac-	X	X	X	X	X	X	D	K	X	X	-NH2
33 Ac-	X	X	X	X	X	X	D	P	X	X	-NH2
34 Ac-	X	X	X	X	X	X	G	D	X	X	-NH2
35 Ac-	X	X	X	X	X	X	G	G	X	X	-NH2
36 Ac-	X	X	X	X	X	X	G	I	X	X	-NH2
37 Ac-	X	X	X	X	X	X	G	K	X	X	-NH2
38 Ac-	X	X	X	X	X	X	G	P	X	X	-NH2
39 Ac-	X	X	X	X	X	X	K	D	X	X	-NH2
40 Ac-	X	X	X	X	X	X	K	G	X	X	-NH2
41 Ac-	X	X	X	X	X	X	K	I	X	X	-NH2
42 Ac-	X	X	X	X	X	X	K	K	X	X	-NH2
43 Ac-	X	X	X	X	X	X	K	P	X	X	-NH2
44 Ac-	X	X	X	X	X	X	P	D	X	X	-NH2
45 Ac-	X	X	X	X	X	X	P	G	X	X	-NH2
46 Ac-	X	X	X	X	X	X	P	I	X	X	-NH2
47 Ac-	X	X	X	X	X	X	P	K	X	X	-NH2
48 Ac-	X	X	X	X	X	X	P	P	X	X	-NH2
49 Ac-	X	X	X	X	X	X	X	X	D	K	-NH2
50 Ac-	X	X	X	X	X	X	X	X	D	P	-NH2
51 Ac-	X	X	X	X	X	X	X	X	D	S	-NH2
52 Ac-	X	X	X	X	X	X	X	X	D	Y	-NH2
53 Ac-	X	X	X	X	X	X	X	X	K	K	-NH2
54 Ac-	X	X	X	X	X	X	X	X	K	P	-NH2
55 Ac-	X	X	X	X	X	X	X	X	K	S	-NH2
56 Ac-	X	X	X	X	X	X	X	X	K	Y	-NH2

Position	1	2	3	4	5	6	7	8	9	10	
Amino acid	A	G	G	G	D	D	D	D	D	K	
	Y	Y	P	I	G	G	G	G	K	P	
			Y	P	P	K	K	I		S	
				Y	Y		P	K		Y	
								P			
57 Ac-	X	G	G	X	X	X	X	X	X	X	-NH2
58 Ac-	X	G	P	X	X	X	X	X	X	X	-NH2
59 Ac-	X	G	Y	X	X	X	X	X	X	X	-NH2
60 Ac-	X	Y	G	X	X	X	X	X	X	X	-NH2
61 Ac-	X	Y	P	X	X	X	X	X	X	X	-NH2
62 Ac-	X	Y	Y	X	X	X	X	X	X	X	-NH2
63 Ac-	X	X	X	G	D	X	X	X	X	X	-NH2
64 Ac-	X	X	X	G	G	X	X	X	X	X	-NH2
65 Ac-	X	X	X	G	P	X	X	X	X	X	-NH2
66 Ac-	X	X	X	G	Y	X	X	X	X	X	-NH2
67 Ac-	X	X	X	I	D	X	X	X	X	X	-NH2
68 Ac-	X	X	X	I	G	X	X	X	X	X	-NH2
69 Ac-	X	X	X	I	P	X	X	X	X	X	-NH2
70 Ac-	X	X	X	I	Y	X	X	X	X	X	-NH2
71 Ac-	X	X	X	P	D	X	X	X	X	X	-NH2
72 Ac-	X	X	X	P	G	X	X	X	X	X	-NH2
73 Ac-	X	X	X	P	P	X	X	X	X	X	-NH2
74 Ac-	X	X	X	P	Y	X	X	X	X	X	-NH2
75 Ac-	X	X	X	Y	D	X	X	X	X	X	-NH2
76 Ac-	X	X	X	Y	G	X	X	X	X	X	-NH2
77 Ac-	X	X	X	Y	P	X	X	X	X	X	-NH2
78 Ac-	X	X	X	Y	Y	X	X	X	X	X	-NH2
79 Ac-	X	X	X	X	D	D	X	X	X	X	-NH2
80 Ac-	X	X	X	X	X	D	G	X	X	X	-NH2
81 Ac-	X	X	X	X	X	D	K	X	X	X	-NH2
82 Ac-	X	X	X	X	X	D	P	X	X	X	-NH2
83 Ac-	X	X	X	X	X	G	D	X	X	X	-NH2
84 Ac-	X	X	X	X	X	G	G	X	X	X	-NH2
85 Ac-	X	X	X	X	X	G	K	X	X	X	-NH2
86 Ac-	X	X	X	X	X	G	P	X	X	X	-NH2
87 Ac-	X	X	X	X	X	K	D	X	X	X	-NH2
88 Ac-	X	X	X	X	X	K	G	X	X	X	-NH2
89 Ac-	X	X	X	X	X	K	K	X	X	X	-NH2
90 Ac-	X	X	X	X	X	K	P	X	X	X	-NH2
91 Ac-	X	X	X	X	X	X	X	D	D	X	-NH2
92 Ac-	X	X	X	X	X	X	X	D	K	X	-NH2
93 Ac-	X	X	X	X	X	X	X	G	D	X	-NH2
94 Ac-	X	X	X	X	X	X	X	G	K	X	-NH2
95 Ac-	X	X	X	X	X	X	X	I	D	X	-NH2
96 Ac-	X	X	X	X	X	X	X	I	K	X	-NH2
97 Ac-	X	X	X	X	X	X	X	K	D	X	-NH2
98 Ac-	X	X	X	X	X	X	X	K	K	X	-NH2
99 Ac-	X	X	X	X	X	X	X	P	D	X	-NH2
100 Ac-	X	X	X	X	X	X	X	P	K	X	-NH2
101 Ac-	A	X	X	X	X	X	X	X	X	K	-NH2
102 Ac-	A	X	X	X	X	X	X	X	X	P	-NH2
103 Ac-	A	X	X	X	X	X	X	X	X	S	-NH2
104 Ac-	A	X	X	X	X	X	X	X	X	Y	-NH2
105 Ac-	Y	X	X	X	X	X	X	X	X	K	-NH2
106 Ac-	Y	X	X	X	X	X	X	X	X	P	-NH2
107 Ac-	Y	X	X	X	X	X	X	X	X	S	-NH2
108 Ac-	Y	X	X	X	X	X	X	X	X	Y	-NH2
109 Ac-	Y	X	X	X	X	X	X	X	X	X	-NH2
110 Ac-	X	G	X	X	X	X	X	X	X	X	-NH2
111 Ac-	X	Y	X	X	X	X	X	X	X	X	-NH2
112 Ac-	X	X	P	X	X	X	X	X	X	X	-NH2
113 Ac-	X	X	X	Y	X	X	X	X	X	X	-NH2
114 Ac-	X	X	X	X	D	X	X	X	X	X	-NH2
115 Ac-	X	X	X	X	X	G	X	X	X	X	-NH2
116 Ac-	X	X	X	X	X	X	K	X	X	X	-NH2
117 Ac-	X	X	X	X	X	X	X	K	X	X	-NH2
118 Ac-	X	X	X	X	X	X	X	X	K	X	-NH2
119 Ac-	X	X	X	X	X	X	X	X	X	S	-NH2

Table 3. IC_{50} for peptides derived from dual-defined PS-SCLs.

	IC_{50} (nM)
IGRRNM-NH_2	30
IGRRYC-NH_2	338
IGRRCY-NH_2	1,083
IGRRHR-NH_2	1,154
IGRRHH-NH_2	1,824

Table 4

Final Sequences from
Iteration of H-XXRRXX-NH_2

I	G	R	R	Y	C
L					L
M					Y
I	G	R	R	E	M
L					W
M					Y

	IC_{50} nM
M G R R Y Y - NH_2	27.5
M G R R Y L - NH_2	28.4
I G R R Y Y - NH_2	29.5
I G R R Y L - NH_2	35.2
I G R R E Y - NH_2	55.8
I G R R E M - NH_2	58.1
M G R R E M - NH_2	60.6
M G R R E Y - NH_2	75.6
L G R R Y Y - NH_2	86.1
M G R R E W - NH_2	190.5
I G R R Y C - NH_2	239.5
L G R R Y L - NH_2	241.7
L G R R E Y - NH_2	241.9
M G R R Y C - NH_2	296.8
I G R R E W - NH_2	313.4
L G R R E Y - NH_2	686.7
L G R R E W - NH_2	972.4
L G R R Y C - NH_2	1,340.7
IGRGNFGEVFSGC-NH_2	152.7

Table 5. IC_{50} values for heptapeptides analogs of IGRRYY-NH_2

	IC_{50} (nM)
IGRRYYD-NH_2	9.0
IGRRYYI-NH_2	11.2
IGRRYYE-NH_2	11.4
IGRRYYG-NH_2	11.6
IGRRYYA-NH_2	14.9
IGRRYYV-NH_2	15.9
IGRRYYQ-NH_2	16.0
IGRRYYF-NH_2	16.6
IGRRYYT-NH_2	17.8
IGRRYYH-NH_2	17.9
IGRRYYP-NH_2	19.5
IGRRYYN-NH_2	19.8
IGRRYYK-NH_2	20.2
IGRRYYM-NH_2	22.8
IGRRYYC-NH_2	23.6
IGRRYYS-NH_2	26.5
IGRRYYL-NH_2	27.6
IGRRYYR-NH_2	29.4
IGRRYYW-NH_2	35.2
IGRRYYY-NH_2	43.5

Table 6 Antigenic determinants identified using omission analogs

mAb 127/LMEQCWAYEPGQRPSF

53F8	-YEPGQR-
42C11	-WAYEPGQ-
50D4	-WAYEPGQ-

mAb 171/IMVKCWMIMDADSRPKF

19B10	-MIDADSR-
11B9	-IDAD-
10E5	-MIDADSR-

Mapping the specificity of an antibody against an oncogenic sequence using peptide combinatorial libraries and substitution analogs: Implications for breast cancer detection

J.R. Appel, J. Buencamino, R.A. Houghten, and C. Pinilla

Torrey Pines Institute for Molecular Studies
3550 General Atomics Ct., San Diego, CA 92121 U.S.A.

Introduction

Proteins encoded by oncogenes, such as *c-erbB-2*, contain a consensus region that has homology with growth factor receptors and protein kinases. These proteins are known to be implicated in breast cancer by their presence in clinical samples of cancer patients. We are studying the specificities of a number of MAbs raised against this consensus region. A better understanding of the aa level specificity of these MAbs will aid in their use as selective probes for early breast cancer detection. Here we have characterized the specificity of a MAb raised against a synthetic peptide from this consensus region using individual substitution analogs and peptide combinatorial libraries.

Results and Discussion

A hexapeptide positional scanning combinatorial library was prepared using a predetermined ratio of aa for each mixture coupling [1] in conjunction with simultaneous multiple peptide synthesis (SMPS) [2]. Hexapeptides derived from the library were synthesized on a COMPAS 242 multiple peptide synthesizer. Individual substitution analogs of the control peptide LGSGAFGTIYKG(C), corresponding to residues 138-149 of the oncogene *v-erbB*, were prepared by SMPS. Each residue of the antigenic determinant, which was identified as -AFGTIYKG- using omission analogs [3], was substituted with one of the other 19 L-aa. Competitive ELISA [4] was used to screen the hexapeptide library, subsequent hexapeptides, and the set of individual substitution analogs against MAb 172-12A4.

The concentration of each substitution analog necessary to inhibit 50% (IC_{50}) of MAb 172-12A4 binding to the control peptide adsorbed to the plate was determined. IC_{50} values for the 19 analogs at each position were averaged. This averaged IC_{50} value is termed the relative positional importance factor (RPIF) and represents the overall replaceability of a given position in the antigenic determinant [4]. It was found that mAb 172-12A4 recognized a discontinuous linear determinant, in which four residues were specific and four residues were relatively redundant. The specific residues were

phenylalanine (RPIF=40.4), the next residue glycine (RPIF=21.4), isoleucine (RPIF=5.5), and lysine (RPIF=20.3). Interestingly, glycine appears twice in the antigenic determinant, once as a specific residue and again as a redundant residue. This pattern of specificity was also found for the relatively conservative aa phenylalanine (specific residue) and tyrosine (redundant residue).

The positional scanning screening data yielded the most active aa residues at each position of the hexapeptide sequence (Table 1). This screening data was sufficient to locate the specific residues of the antigenic determinant, namely positions 2-7 since alanine and glycine at positions 1 and 8, respectively, were redundant. Tyrosine in position 1 of the library was nearly five-fold better than the expected phenylalanine and represents a conservative substitution. Sixteen individual peptides derived from the combinations of the most active aa at each position of the library were synthesized and assayed. Peptides having proline at the third position were at least 10-fold more active than those having glycine at the same position. Also, peptides having tyrosine at the second position were poorly recognized, indicating that the peptides responsible for the activity found for Ac-XYXXXX-NH_2 are not the same as those in Ac-YXXXXX-NH_2.

Peptide combinatorial libraries and individual substitutiuon analogs reveal similar patterns of specificity for peptide-antibody interactions. A complete set of substitution analogs yielded a "fingerprint" profile for this peptide-antibody interaction as well as the relative importance of each antigenic determinant residue. The screening data of the positional scanning library revealed a number of high affinity sequences. Finally, a single positional scanning combinatorial library can be used to study a number of different antigen-antibody interactions.

Table 1 *Library screening data and activities of peptides recognized by MAb 172-12A4*

Library	Active residues	Peptide	IC_{50} (μM)
Ac-O_1XXXXX-NH_2	Y	Ac-YGPIDK-NH_2	1.2
Ac-XO_2XXXX-NH_2	G, Y	Ac-YGPIPK-NH_2	4.1
Ac-XXO_3XXX-NH_2	G, P	Ac-YGPIIK-NH_2	5.5
Ac-$XXXO_4$XX-NH_2	I	Ac-YGPIKK-NH_2	6.1
Ac-$XXXXO_5$X-NH_2	D, I, K, P	Ac-YGGIDK-NH_2	15
Ac-$XXXXXO_6$-NH_2	K	Ac-YGGIPK-NH_2	75
		all other peptides	>100

Acknowledgments

This work was funded by grant DAMD17-94-J-4110 from USAMRDC.

References

1. Pinilla, C., Appel, J.R., Blanc, P., and Houghten, R.A. BioTechniques, 13 (1992) 901.
2. Houghten, R.A. Proc. Natl. Acad. Sci., 82 (1985) 5131.
3. Appel, J.R., Pinilla, C., Niman, H., and Houghten, R.A. J. Immunol., 144 (1990) 976.
4. Pinilla, C., Appel, J.R., and Houghten, R.A. Mol. Immunol., 30 (1993) 577.

Exploring antibody polyspecificity using synthetic combinatorial libraries

Jon R. Appel, Jaime Buencamino, Richard A. Houghten, and Clemencia Pinilla

Torrey Pines Institute for Molecular Studies
3550 General Atomics Court
San Diego, CA 92121, U.S.A.

Corresponding author:

Clemencia Pinilla
Phone: 619-455-3843
Fax: 619-455-3804
e-mail: pinilla@tpims.com

Keywords: synthetic combinatorial libraries, positional scanning, monoclonal antibodies, polyspecificity

Summary

Extensive mapping studies for seven antigen-antibody interactions have been carried out using both individual analogs and peptide libraries. With competitive ELISA, these studies have revealed that monoclonal antibodies exhibit a broad range of specificities, from antibodies that recognize only conservative substitutions for 1-2 positions of the antigenic determinant to antibodies that recognize sequences that are completely unrelated to the parent antigen with comparable affinities. Synthetic combinatorial libraries, containing millions of peptide sequences, permit a more systematic and rapid evaluation of the extent of multiple binding specificities of monoclonal antibodies than individual analogs. The peptide libraries used here are comprised of mixtures of compounds having specifically defined positions and mixture positions. The same diversity of sequences in different formats, which differ by the numbers of positions singularly defined and different locations defined within the sequence, can be examined. Comparison of the screening results, selection criteria of the most active mixtures, and different approaches used for deconvolution of active individual compounds are discussed. Synthetic combinatorial libraries greatly facilitate the understanding of antigen-antibody interactions at the amino acid level and will assist in the development of improved immunodiagnostics.

Introduction

A better understanding of the fine specificity of antibody binding to synthetic peptides at the amino acid level will aid in the development of improved immunodiagnostics. Peptide analogs and combinatorial libraries of peptides have proven to be useful tools that yield similar patterns of specificity for peptide-antibody interactions. One advantage of using combinatorial libraries over analogs is that one peptide library can be used to study the binding specificities of many different antibodies. Furthermore, antigen-antibody studies using various peptide library approaches [1-7] have shown that different monoclonal antibodies are able to recognize peptide sequences unrelated to the immunogen (termed polyspecificity), which would be extremely difficult, if not impossible, to do with peptide analogs. These studies support the idea that antibodies can be functionally polyspecific, in which an antibody's binding site can recognize antigens that differ by more than one amino acid residue [8]. For the development of improved immunodiagnostics all that is required for a synthetic mimic of an antigenic determinant is a sufficient degree of antigen similarity for significant antibody binding. Peptide libraries, made up of enormous diversities of peptide sequences, offer the unique opportunity to accelerate the systematic study of antibody polyspecificity.

Synthetic combinatorial libraries (SCLs) are composed of mixtures of compounds having defined positions and mixture positions, as reviewed in [9]. More than one library can represent the same diversity depending on the number of positions defined and the location of the defined positions within the sequence. This study summarizes the results found in the mapping of several different antigen-antibody interactions using dual-defined SCLs and positional scanning SCLs [10,11]. The different approaches used for deconvolution of active individual sequences will be discussed.

Materials and Methods

Library synthesis

Dual-defined and positional scanning SCLs were prepared as previously described [10,11]. Dual-defined libraries contain two positions defined with one of the 20 L-amino acids (represented as O) and four positions as mixtures of amino acids (represented as X). Three different dual-defined libraries (same diversity, different formats) have been prepared and can be represented as $O_1O_2XXXX\text{-}NH_2$, $XXO_3O_4XX\text{-}NH_2$, and $XXXXO_5O_6\text{-}NH_2$ yielding twelve hundred different peptide mixtures representing a diversity of 52 million hexapeptide sequences. Positional scanning SCLs contain one position defined and the remaining positions as mixtures. For a hexapeptide positional scanning SCL (represented as: $O_1XXXXX\text{-}NH_2$, $XO_2XXXX\text{-}NH_2$, $XXO_3XXX\text{-}NH_2$, $XXXO_4XX\text{-}NH_2$, $XXXXO_5X\text{-}NH_2$, and $XXXXXO_6\text{-}NH_2$) each positional library consists of 20 peptide mixtures for a library totaling 120. This library also consists of the same diversity, but in a different format from the dual-defined libraries. Briefly, peptide mixtures making up each library were synthesized using a predetermined ratio of protected L-amino acids in the mixture coupling [12,13] in conjunction with simultaneous multiple peptide synthesis [14]. Peptide mixtures were cleaved from their resins, extracted, lyophilized, and reconstituted in water. Individual peptides were synthesized by either SMPS or on a COMPAS (Spyder Instruments, San Diego, CA). Purity and identity of peptides were characterized by RP-HPLC and MALDI mass spectrometry, respectively.

Competitive ELISA

Peptide libraries and individual peptides were screened by competitive ELISA as previously described [15,16]. Peptide or protein antigen was adsorbed to microtiter plates at concentrations previously determined to yield optimal antibody binding. Plates were washed with water and blocked with 1.5%BSA/PBS. Libraries or individual sequences were added to plate

wells in serial dilutions, followed by a previously determined concentration of respective monoclonal antibody. Bound antibody was detected with goat-antimouse HRP conjugate. Plates were developed with OPD/H_2O_2 as substrate and the reaction was stopped with sulfuric acid. Optical densities were measured at 492nm on a Titertek Multiscan. The concentration of each peptide mixture or individual peptide necessary to inhibit 50% (IC_{50}) of the binding of monoclonal antibody to the antigen on the plate was determined.

Results

Using SCLs having the same diversities, but different formats, we have mapped seven antigen-antibody interactions to the amino acid level using competitive ELISA (Table 1). These studies have revealed that monoclonal antibodies exhibit a broad range of specificities. These range from antibodies recognizing only conservative substitutions at 1-2 positions in the antigenic determinant to those that recognize completely unrelated sequences to the parent antigen while having comparable affinities. The identification of unrelated sequences, which are recognized with high binding affinities by monoclonal antibodies, suggests that these antibodies can be considered functionally polyspecific. Also, the screening of SCLs yielded sequences recognized by their respective antibodies with higher affinities than the original immunogen. It should be noted that deconvolution of SCLs to identify individual sequences is primarily dependent on the assay results, and not the format of the library. The following examples will serve to illustrate the different routes one can use to identify individual peptides from the screening results.

Interaction 1

Monoclonal antibody LJ-134B29 was raised against the peptide SSTSYNRGDSTFESK, representing residues 566-580 of fibrinogen A α. A hexapeptide PS-SCL was screened for inhibition of mAb LJ-134B29 binding to fibrinogen [6]. Based on IC_{50} values of the most active mixtures from the screening results, eight peptides were synthesized and assayed. All were recognized by mAb LJ-134B29 with affinities up to 100-fold higher than the immunogen. The peptides derived from the library clearly identified the sequence of the antigenic determinant in the immunogen, as well as single and double substitutions (i.e., Ac-GESTFE-NH_2, IC_{50}=1.5nM, Table 1).

Interaction 2

Monoclonal antibody M1 was raised against the FLAG octapeptide sequence DYKDDDDK and was found to bind to this peptide in a calcium-dependent manner. A hexapeptide PS-SCL was screened for inhibition of mAb M1 binding to the FLAG octapeptide, both in the presence and absence of calcium [5]. Based on the IC_{50} values of the most active peptide mixtures from the screening results, 16 hexapeptides were synthesized and assayed. All were recognized by mAb M1 in the absence of calcium with 50- to 100-fold higher affinities than the FLAG octapeptide. Also, the hexapeptides identified from the library were recognized 10-fold higher than the FLAG octapeptide in the presence of calcium. Interestingly, the fifth position aspartic acid of the FLAG sequence was replaced with an oppositely charged lysine (i.e., DYKAKE-NH_2, IC_{50}=3nM, Table 1). Although the first three residues are the same in the immunogen, the last three residues could not have been found without making a large number of individual multiple substitution analogs.

Interaction 3

Monoclonal antibody 222-35C8 was raised against the peptide LHNNEAGRTTVFS, corresponding to residues 200-212 of the oncogene-related protein from *int-1*. A dual-defined SCL (Ac-O_1O_2XXXX-NH_2) was screened for inhibition of mAb 222-35C8 binding to the immunogen [7]. Three peptide mixtures were found with significant activity, namely Ac-

GRXXXX-NH_2, Ac-RGXXXX-NH_2, and Ac-QEXXXX-NH_2. An iterative synthesis and screening process was used to identify three different families of peptide sequences recognized by mAb 222-35C8. The most active peptides from each family were Ac-GRWTVF-NH_2, Ac-RGIVFP-NH_2, and Ac-QEFHAS-NH_2 (Table 1).

Interaction 4

Monoclonal antibody LJ-155B16 was raised against the peptide TTNIMEILRGDFSS, corresponding to fibrinogen Aα 87-100. A dual-defined SCL (Ac-O_1O_2XXXX-NH_2) was screened for inhibition of mAb LJ-155B16 binding to fibrinogen [6]. An iterative process was carried out on the most active peptide mixture, Ac-WWXXXX-NH_2, to define the remaining mixture positions. The most active sequence, Ac-WWYESW-NH_2 (IC_{50}=40nM, Table 1), was completely unrelated (i.e. had no sequence homology) to the original immunogenic sequence, but was recognized by the mAb with the same affinity. Overlapping hexapeptides of the immunogen were poorly recognized (IC_{50}>100μM). A biotinylated version of this sequence captured with streptavidin in ELISA was tested against several other fibrinogen-related antibodies for direct binding. No significant binding was found, supporting the competitive ELISA results that this sequence is specifically recognized by mAb LJ-155B16.

Interaction 5

Monoclonal antibody 12 was raised against the surface antigen of Hepatitis B virus (HBsAg). A hexapeptide PS-SCL was screened against mAb 12 for inhibition of binding [17]. The peptide Ac-STTSMM-NH_2 (IC_{50}=170nM, Table 1) was identified, in which four residues corresponded to residues 114-117 of HBsAg. One of the most active peptide mixtures from the screening was Ac-XXXPXX-NH_2, although none of the resulting individual peptides containing proline at the fourth position were found within the active peptides. However, the completion of the iterative process from this peptide mixture yielded an active individual peptide (Ac-SVGPPH-NH_2, IC50=170nM, Table 1) that was completely different from the sequences derived from the PS-SCL. When the two sequences were incorporated into one peptide, the activity was equal to HBsAg (IC_{50}=14nM).

Interaction 6

Monoclonal antibody 172-12A4 was raised against the peptide LGSGAFGTIYKG, corresponding to residues 138-149 of the oncogene *v-erbB*. Using omission analogs, it was found that mAb 172-12A4 recognizes an 8-residue determinant, -AFGTIYKG- [18]. A hexapeptide PS-SCL was screened against mAb 172-12A4 for inhibition of binding [19]. The most active sequence, Ac-YGPIDK-NH_2 (IC_{50}=1200 nM, Table 1) contains the motif -YG_I_K-, which is similar to the antigenic determinant. Extending this sequence to seven or eight residues may result in peptides having improved activities.

Interaction 7

Monoclonal antibody 121-15B10 was raised against the peptide IGRGNFGEVFSG representing residues 529-540 of the v-fes oncogene-encoded protein. Omission analog analysis by ELISA mapped the antigenic determinant to the first three residues (-IGR-) of the peptide. A number of differently formatted SCLs were screened for inhibition of mAb 121-15B10 binding to the immunogen peptide by competitive ELISA. Individual sequences were identified from several SCLs using a number of different strategies.

The screening of the length SCL (OXX-NH_2 up to OXXXXXXXXX-NH_2) revealed that isoleucine, and to a lesser extent methionine, were the most active amino acids in the first position of sequences ranging from 6-9 residues in length. A hexapeptide positional scanning SCL was screened, and based on the screening results, individual peptides resulting from the most active amino acids were prepared and assayed. The first three residues of the most active peptides corresponded with the immunogen sequence (-IGR-).

Next, three different dual-defined SCLs were screened. Position 1 was specific for isoleucine, methionine, and leucine, and position two was specific only for glycine. Positions 3 and 4 were specific only for arginine. Peptide mixtures having the fifth and sixth position defined were less active, yet several amino acids were chosen. Based on the IC_{50} values of the most active mixtures, five individual peptides were prepared and tested. The most active peptide was IGRRNM-NH_2 (IC_{50}=30nM, Table 1) in which four of the six residues (IGR_N_) correlate with the immunogen.

The iterative process was also carried out for the most active peptide mixture from the three dual-defined SCLs, namely XXRRXX-NH_2. Using a bidirectional approach, the first set of iterations defined the second and fifth positions. Upon screening these iterations, glycine was clearly the only active amino acid at position 2. Tyrosine and other aromatic amino acids, as well as asparagine, aspartic and glutamic acid, were the most active at position 5, although their activities were only threefold better than XXRRXX-NH_2. The next set of iterations defined the first and sixth positions of XGRRYX-NH_2. Isoleucine and methionine were the most active in position 1, whereas tyrosine and several other amino acids were active in position 6. Final sequences were made from the iterative screening data and assayed. The most active sequences contained the following motif: (I/M) G R R (Y/E) (Y/L/M). The first four residues of this motif were found to be the most specific for this peptide-antibody interaction. These results correlate well with those found from screening the three dual-defined SCLs, indicating that differently formatted libraries representing the same diversities will yield similar results.

Since the length library resulted in peptide mixtures of 6-9 residues that were the most active, heptapeptide analogs were prepared for IGRRYY-NH_2 in an effort to improve its activity. Each of the 20 L-amino acids was added to the C-terminus and assayed. A two- to threefold increase was found for the most active peptide IGRRYYD-NH_2 (IC_{50}=9nM, Table 1).

Discussion

From the above examples, it was found that positional scanning libraries and dual-defined SCLs, representing the same diversities in different formats, complemented each other in the study of antibody specificity. In some cases, one type of library yielded better results, due to positional importance of the residues that were defined, the affinity and specificity of the antigen-antibody interaction, and the differences in individual peptide concentration between positional scanning and dual-defined SCLs. In most cases, the screening of peptide libraries by competitive ELISA yielded sequences that were recognized by their respective antibodies with higher affinities than their immunogen. Individual sequences can be rapidly deconvoluted from each library by either the iterative approach, synthesizing individual peptides directly from the screening data, or a combination of the two. Unrelated sequences were also identified from SCLs and could not have been found using analogs of the immunogen. Specific residues for these antigen-antibody interactions were found at their respective positions within the antigenic sequence in differently formatted SCLs.

As noted, the deconvolution of sequences from a library is contingent on the screening results, as well as the affinity and specificity of the interaction. If the screening results are clear, regarding positional specificity and importance, then individual sequences can be prepared directly from the combinations of amino acids defined in most active peptide mixtures (see interactions 1,2, 5, 6, and 7). For screening results that are less clear, iterations can be carried out on one or more of the most active peptide mixtures to yield individual active sequences (see interaction 5). Iterative deconvolution from an active peptide mixture, while more time-consuming due to the number of separate syntheses required, were successful in the identification of active sequences (see interactions 3, 4, and 7). In earlier work, the positional scanning and iterative deconvolution approaches have been used to identify active sequences recognized by the same monoclonal antibody. These two approaches yielded the same peptide

sequences for mAb 17/9 [10,11] and mAb 3E7 [20,21]. In another example, highly active sequences were deconvoluted using the iterative approach [22] that were not identified from the positional scanning approach [11]. Lastly, the type of deconvolution approach taken will depend on one's level of resources due to the number of sequences that need to be made.

It has been shown that only 3-5 residues of peptide antigens are specifically recognized by monoclonal antibodies [15,23-26]. Redundant residues within the antigenic determinant may be replaced with other amino acids to "improve" their antigenicity [7]. It has been proposed that since antibodies have flexible hypervariable loops, each antibody binding site is not unique for a single ligand, but can be more appropriately described as functionally polyspecific [8]. Antibody polyspecificity therefore can be examined systematically using libraries to search for antigens that are not structurally exact, but have some degree of epitope resemblance. As shown in this study, polyspecificity may be a general feature of antibodies, even for those of high affinity.

The ability to screen SCLs in solution at different concentrations facilitates the selection of the most active mixtures, which ultimately leads to the most active sequences from the library. The strategies for deconvolution of active sequences from SCLs, as reviewed here, have also been applied to peptidomimetic and organic SCLs arranged in similar formats [27-29].

Acknowledgements

This work was supported by Houghten Pharmaceuticals, Inc., San Diego, CA and by grant DAMD17-94-J-4110 from USAMRDC.

References

1 Christian, R.B., Zuckermann, R.N., Kerr, J.M., Wang, L. and Malcolm, B.A. , Simplified methods for construction, assessment and rapid screening of peptide libraries in bacteriophage, J. Mol. Biol. 227 (1992) 711-718.

2 Felici, F., Castagnoli, L., Musacchio, A., Jappelli, R. and Cesareni, G. , Selection of antibody ligands from a large library of oligopeptides expressed on a multivalent exposition vector, J. Mol. Biol. 222 (1991) 301-310.

3 Needels, M.C., Jones, D.G., Tate, E.H., Heinkel, G.L., Kochersperger, L.M., Dower, W.J., Barrett, R.W. and Gallop, M.A. , Generation and screening of an oligonucleotide-encoded synthetic peptide library, Proc. Natl. Acad. Sci. USA, 90 (1993) 10700-10704.

4 Scott, J.K. and Smith, G.P. , Searching for Peptide Ligands with an Epitope Library, Science, 249 (1990) 386-390.

5 Pinilla, C., Buencamino, J., Appel, J.R., Hopp, T.P. and Houghten, R.A. , Mapping the detailed specificity of a calcium-dependent monoclonal antibody through the use of soluble positional scanning combinatorial libraries: Identification of potent calcium-independent antigens, Mol. . Diversity, 1 (1995) 21-28.

6 Pinilla, C., Buencamino, J., Appel, J.R., Houghten, R.A., Brassard, J.A. and Ruggeri, Z.M. , Two antipeptide monoclonal antibodies that recognize adhesive sequences in fibrinogen: Identification of antigenic determinants and unrelated sequences using synthetic combinatorial libraries, Biomedical. Peptides,. Proteins. &. Nucleic. Acids, 1 (1995) 199-204.

7 Pinilla, C., Chendra, S., Appel, J.R. and Houghten, R.A. , Elucidation of monoclonal antibody polyspecificity using synthetic combinatorial library, Pept. Res. 8 (1995) 250-257.

8 Van Regenmortel, M.H.V. , Transcending the structuralist paradigm in immunology-affinity and biological activity rather than purely structural considerations should guide the design of synthetic peptide epitopes, Biomedical. Peptides,. Proteins. &. Nucleic. Acids, 1 (1995) 109-116.

9 Pinilla, C., Appel, J., Blondelle, S.E., Dooley, C.T., Dörner, B., Eichler, J., Ostresh, J.M. and Houghten, R.A. , A review of the utility of peptide combinatorial libraries, Biopolymers. (Peptide. Science), 37 (1995) 221-240.

10 Houghten, R.A., Pinilla, C., Blondelle, S.E., Appel, J.R., Dooley, C.T. and Cuervo, J.H. , Generation and use of synthetic peptide combinatorial libraries for basic research and drug discovery, Nature, 354 (1991) 84-86.

11 Pinilla, C., Appel, J.R., Blanc, P. and Houghten, R.A. , Rapid identification of high affinity peptide ligands using positional scanning synthetic peptide combinatorial libraries, Biotechniques, 13 (1992) 901-905.

12 Ostresh, J.M., Winkle, J.H., Hamashin, V.T. and Houghten, R.A. , Peptide libraries: Determination of relative reaction rates of protected amino acids in competitive couplings, Biopolymers, 34 (1994) 1681-1689.

13 Dooley, C.T. and Houghten, R.A. , The use of positional scanning synthetic peptide combinatorial libraries for the rapid determination of opioid receptor ligands, Life. Sci. 52 (1993) 1509-1517.

14 Houghten, R.A. , General method for the rapid solid-phase synthesis of large numbers of peptides: Specificity of antigen-antibody interaction at the level of individual amino acids, Proc. Natl. Acad. Sci. USA, 82 (1985) 5131-5135.

15 Pinilla, C., Appel, J.R. and Houghten, R.A. , Functional importance of amino acid residues making up peptide antigenic determinants, Mol. Immunol. 30 (1993) 577-585.

16 Pinilla, C., Appel, J.R. and Houghten, R.A. , Identification of antigenic determinants using synthetic peptide combinatorial libraries, In Coligan, J.E., Kruisbeek, A.M., Margulies, D.H., Shevach, E.M. and Strober, W. (Eds.) Current Protocols in Immunology, John Wiley & Sons,Inc, New York, 1994, pp. 9.8.1-9.8.15.

17 Pinilla, C., Buencamino, J., Houghten, R.A. and Appel, J.R. , Detailed studies of antibody specificity using synthetic combinatorial libraries, In Brown, F., Chanock, R.M., Ginsberg, H.S. and Norrby, E. (Eds.) Vaccines 1995: Molecular Approaches to the Control of Infectious Diseases, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1995, pp. 13-17.

18 Appel, J.R., Pinilla, C., Niman, H. and Houghten, R.A. , Elucidation of discontinuous linear determinants in peptides, J. Immunol. 144 (1990) 976-983.

19 Appel, J.R., Buencamino, J., Houghten, R.A. and Pinilla, C. , Mapping the specificity of an antibody against an oncogenic sequence using peptide combinatorial libraries and substitution analogs: Implications for breast cancer detection, In Kaumaya, P. (Eds.) Peptides, Proceedings of the 14th American Peptide Symposium, 1996,

20 Pinilla, C., Appel, J.R. and Houghten, R.A. , Synthetic peptide combinatorial libraries (SPCLs): Identification of the antigenic determinant of β-endorphin recognized by monoclonal antibody 3E7, Gene, 128 (1993) 71-76.

21 Pinilla, C., Appel, J.R., Blondelle, S.E., Dooley, C.T., Eichler, J., Ostresh, J.M. and Houghten, R.A. , Versatility of positional scanning synthetic combinatorial libraries for the identification of individual compounds, Drug. Dev. Res. 33 (1994) 133-145.

22 Appel, J.R., Pinilla, C. and Houghten, R.A. , Identification of related peptides recognized by a monoclonal antibody using a synthetic peptide combinatorial library, Immunomethods, 1 (1992) 17-23.

23 Getzoff, E.D., Tainer, J.A., Lerner, R.A. and Geysen, H.M. , The chemistry and mechanism of antibody binding to protein antigens, Adv. Immunol. 43 (1988) 1-98.

24 Geysen, H.M., Mason, T.J. and Rodda, S.J. , Cognitive features of continuous antigenic determinants, J. Mol. Recog. 2 (1989) 32-49.

25 Rini, J.M., Schulze-Gahmen, U. and Wilson, I.A. , Structural evidence for induced fit as a mechanism for antibody-antigen recognition, Science, 255 (1992) 959-965.

26 Churchill MEA, Stura E, Pinilla C, Appel JR, Houghten RA, Kono DH, Balderas RS, Fieser GG, Schulze-Gahmen U, and Wilson IA. Crystal structure of a peptide complex of anti-influenza peptide antibody Fab 26/9: Comparison of two different antibodies bound to the same peptide antigen. J Mol Biol 1994;241534-56.

27 Ostresh, J.M., Husar, G.M., Blondelle, S.E., Dörner, B., Weber, P.A. and Houghten, R.A. , "Libraries from libraries": Chemical transformation of combinatorial libraries to extend the range and repertoire of chemical diversity, Proc. Natl. Acad. Sci. USA, 91 (1994) 11138-11142.

28 Dooley, C.T. and Houghten, R.A. , Identification of mu-selective polyamine antagonists from a synthetic combinatorial library, Analgesia, 1 (1995) 400-404.

29 Eichler, J., Appel, J.R., Blondelle, S.E., Dooley, C.T., Dörner, B., Ostresh, J.M., Pérez-Payá, E., Pinilla, C. and Houghten, R.A. , Peptide, peptidomimetic and organic synthetic combinatorial libraries, Med. Res. Rev. 15 (1995) 481-496.

Table 1

Peptide sequences recognized by monoclonal antibodies identified from SCLs.

mAb	SCL used	Sequence	IC_{50} (nM)	Ref.
LJ-134B29	hexa-PS-SCL	Ac-GESTFE-NH_2	1.5	[6]
M1	hexa-PS-SCL	DYKAKE-NH_2	3	[5]
222-35C8	Ac-O_1O_2XXXX-NH_2	Ac-GRWTVF-NH_2 Ac-RGIVFP-NH_2 Ac-QEFHAS-NH_2	2 4 68	[7]
LJ-155B16	Ac-O_1O_2XXXX-NH_2	Ac-WWYESW-NH_2	40	[6]
12	hexa-PS-SCL	Ac-STTSMM-NH_2 Ac-SVGPPH-NH_2	170 170	[17]
172-12A4	hexa-PS-SCL	Ac-YGPIDK-NH_2	1200	[19]
121-15B10	XXO_3O_4XX-NH_2	IGRRNM-NH_2 IGRRYYD-NH_2	30 9	here